VOL. XI, No. 4 MAY, 1950

BORDEN'S REVIEW

of

NUTRITION RESEARCH

THE UTILIZATION OF DIETARY PROTEIN

Protein Supplementation

BORDEN'S REVIEW
of
NUTRITION RESEARCH

Reviews and summaries of research information concerning nutrition and foods. Published monthly, except July and August, by THE BORDEN COMPANY; officers of the Company: Theodore G. Montague, president; E. L. Noetzel, treasurer; T. D. Waibel, secretary. Editorial office: 350 Madison Avenue, New York 17, N. Y.

THE UTILIZATION OF DIETARY PROTEIN

Protein Supplementation

By RUTH WOODS

Long before the coming of age of nutrition as a new member of the family of biological sciences, studies on the nature and functions of foodstuffs established the unique dietary importance of the nitrogen-containing materials which we now call the proteins. With the parallel advances in nutrition, biochemistry, physiology, cytology and numerous other related sciences, it became possible to piece together findings from these separate fields to create the story of the proteins and their role in the nutrition of the living organism. From the elementary fact that proteins are a vital constituent of the diet, the story of protein nutrition has advanced rapidly. It has now been established that the proteins constitute the very heart of living matter, being the stuff of which protoplasm is formed. They serve also, in the form of nucleoproteins and enzymes, as the very activators of vital processes such as reproduction; cell growth, maturation and differentiation; virus activity; genetic activity and the like.

With the growing awareness of the special importance of proteins came the realization that not all proteins are alike in their ability to contribute essential materials to the body. This realization stemmed from the pioneer researches of Osborne and Mendel on the amino acid composition of proteins and from the subsequent studies of Rose and coworkers distinguishing between the so-called "essential" and "non-essential" amino acids. As protein biochemistry has become refined into amino acid biochemistry, it has become possible to make more and more discoveries concerning the behavior and importance of these materials in nutrition.

A direct outgrowth of amino acid research has been the discovery of the phenomenon of protein supplementation. Although this phenomenon is so obviously self-evident and has, indeed, been recognized in one way or another for many years, there are certain aspects of protein supplementation which have only recently been uncovered. Perhaps the most important of these is the time element as a limiting factor in the efficacy of protein supplementation. These new studies are of the utmost significance in practical dietetics and

will surely necessitate considerable revision in present methods of menu construction.

The Amino Acid Make-up of Proteins:

The key to the phenomenon of supplementation lies in an understanding of the nutritional significance of the various amino acids. This subject has been reviewed in detail in previous issues and will be repeated here only briefly for purposes of orientation.

There are some twenty-two amino acids known which, in various combinations, go to make up the proteins. Although more than half of the twenty-two amino acids had been discovered before 1900, very little was known concerning their individual functions and actual distribution in various food proteins. Therefore, it was not at first recognized that the nutritive value of a protein depends upon the nature of its amino acid constituents. Attention was focused on total protein intake rather than on the quality or type of protein eaten. As new chemical methods for the separation of the various classes of amino acids became available, knowledge concerning protein chemistry advanced rapidly. The relationship between amino acid composition and the nutritive adequacy of individual proteins finally became apparent. It was soon appreciated that fundamental knowledge of protein metabolism could be obtained only through a knowledge of the behavior and biological significance of the individual amino acids.

The "Essential" Amino Acids: For this reason, scientific attention was directed from about 1912 to 1930 to finding out which of the known amino acids are indispensable and which may safely be excluded from the diet without fatal consequences, or without impairing growth. Not until the researches of Rose (1) during 1930-1938, however, was the final answer to this problem attained. As a result of these researches, it was established that only ten amino acids are required by the rat for growth. These ten were classed as the so-called "essential" amino acids, essential in the sense that they must be provided preformed in the diet. The other twelve amino acids are, of course, essential for the rat for various biochemical reactions, but they can be synthesized within the organism. Later studies with human subjects established the fact that only eight of the ten "essentials" for rats are necessary for adult men (Table 1). The criterion used for human subjects was, unlike that in rats, not growth but rather *maintenance,* measured in terms of nitrogen balance (2).

The Nutritive Value of Proteins:

The newer knowledge of the biological differences among the amino acids led to the classification of proteins as biologically adequate or complete or biologically incomplete. A biologically complete protein is one which contains all of the so-called "essential" amino acids. A biologically incomplete protein is deficient in one or more of these amino acids. This deficiency may be either absolute or relative. In other words, some proteins may lack almost entirely one or more amino acid; others may contain all the essential amino acids, but certain ones may be present in such limited amounts as to render the protein "incomplete." This somewhat elementary distinction between proteins was soon followed by more precise methods of determining nutritive values. These methods were of two basic types: biological and chemical.

Table 1. Essential Amino Acids.

Amino Acid	For Growth In Rat*	For Maintenance of Nitrogen Balance In Adult Man: Minimum Daily Requirement	For Maintenance of Nitrogen Balance In Adult Man: Recommended Daily Intake
		grams	grams
L-Tryptophan	✓	0.25	0.5
L-Phenylalanine	✓	1.10	2.2
L-Lysine	✓	0.80	1.6
L-Threonine	✓	0.50	1.0
L-Valine	✓	0.80	1.6
L-Methionine	✓	1.10	2.2
L-Leucine	✓	1.10	2.2
L-Isoleucine	✓	0.70	1.4
L-Arginine	✓†	—	—
L-Histidine	✓	—	—

*Rose has investigated the relative nutritive values of the optical isomers of the essential amino acids (except arginine). He reports (1) that only the naturally occurring forms of valine, leucine, isoleucine, lysine and threonine are effective for the rat, whereas both isomers of tryptophan, phenylalanine, methionine and histidine are used. Man apparently utilizes the natural L- forms, although both D- and L-methionine are equally well utilized and, to a certain extent, D-phenylalanine may also be used.

†Arginine can be synthesized by the rat, but not rapidly enough to meet the demands of normal growth. It is conceivable, therefore, that while the human adult does not require this amino acid, it may be essential for growing children and adolescents. This has not yet been investigated.

The Biological Estimation of Protein Quality: Numerous methods based on biological tests with experimental animals have been devised in order to determine the nutritive value of a given protein. Of these, the two following have been most widely used.

A. *(The Protein Efficiency Ratio):* This method was originally proposed by Osborne *et al* (4) and has since been modified by others (5, 6). It is designed to rate protein nutritive quality in terms of the efficiency with which

it is used. "Efficiency of use" is determined by correlating the gain in weight of the animal with the number of grams of protein consumed. This method suffers from several shortcomings. The most apparent of these is the fact that the ratio of gain in weight to total protein consumed is not a true efficiency ratio because not all of the protein eaten is used for growth; only that consumed *above* basic requirements for maintenance.

B. *(Biological Value and Net Utilization Method):* This method is based upon data referring to nitrogen metabolism and involves an estimation of both digestibility and degree of metabolic utilization (7). The "biological value" measures the percentage of absorbed nitrogen retained for growth and for the replenishment of endogenous losses (in the rat). This value, multiplied by the coefficient of digestion and divided by 100 gives a figure which represents the "net" utilization of a particular dietary protein. The method has been improved several times (8, 9) and many modifications of the technique are in use. The results of these methods have been carefully tested for validity (10, 11, 12) and, with minor failings, the general method is believed to be fairly accurate.

The Chemical Estimation of Protein Quality: This method is based on a quantitative analysis of the amino acid makeup of a given protein. By itself this method could not originally afford a sound basis of judgment of the nutritive value of a protein or even of the amino acid limiting its nutritive value. This is because the quantitative amino acid requirements of the test animal (rat) were, and still are, unknown.* For man, however, tentative figures have recently been determined (2) making it possible to draw definite conclusions from an estimation of amino acid values† of various proteins. The original manner of using this method, therefore, was based on a comparison of the amino acid makeup of the test protein with that of a standard reference material (13)—i.e. whole egg protein, (including the proteins of both egg yolk and egg white) chosen because of its proven high digestibility and almost perfect utilization (8, 14). Assigning whole egg protein a value of 100, it was possible to assign numerical score values to other proteins and to determine which of the amino acids in each was the "limiting" factor in the nutritive value of the protein. In most cases, these ratings have correlated very closely with biological evaluations (15).

A new, chemical method for estimating protein quality in terms of comparison with the amino acids of whole egg protein was announced by Oser

*See, however, section below.

†Here again, account must be taken of the reliability of existing methods for the determination of amino acids and of the changing values which may result from the development of improved analytical techniques.

(15a) in April of this year. He has suggested a method for arriving at a biological evaluation of the essential amino acid contribution of food proteins in terms of an "Essential Amino Acid (EAA) Index." This value is closely correlated with (and, in fact, is numerically almost equal to) the biological value in the Mitchell sense. The EAA Index is the geometric mean of the percentage ratios of ten essential amino acids in the food protein (N x 6.25) compared to their respective contents in whole egg protein, assumed to be a nutritionally "ideal" protein. The EAA Index is easily computed by dividing the sum of the logarithms of the ten "egg ratios" by ten and taking the antilogarithm of this mean. The EAA Index thus correlates the amino acid composition as determined analytically with a biological function of the protein. It makes possible predictions of the quantitative effect of supplementation with amino acids or with other proteins.

The Importance of Supplementation in Practical Dietetics:

In commenting on the significance of the amino acid analysis method as a means of determining the nutritive value of a protein, Block and Mitchell (15) point out the immense practical importance of supplementation among food proteins.

An outstanding advantage of this method over biological evaluation, according to these investigators, concerns the supplementary relations existing among food proteins, whereby amino acid deficiencies in one protein mixture are corrected by amino acid excesses in another and *vice versa.* Such relationships are, of course, mutual and always exist where food proteins possessing different amino acid deficiencies are combined into a diet. Block and Mitchell emphasize the fact that *"practical nutrition is concerned with the nutritive value of diets, not of individual foods."* The classification of individual food proteins as good, bad or indifferent in covering the body's requirements when fed alone is of little significance in assessing the protein values of diets, because of these supplementary relationships.

Such relationships cannot be detected by biological methods since biological values (based on growth response, utilization, etc.) give no indication as to how the test protein may be improved by proper amino acid supplementation. The complete amino acid analysis of a food, as illustrated in Table 2, makes it possible to predict the first, as well as subsequent, limiting essential amino acid in each case. Such information, it is further pointed out, reveals clearly the supplementary relations among proteins and affords a valuable guide to the dietitian in compounding protein mixtures of the greatest nutritional efficiency.

Table 2. The Percentage Deviations of the Essential Amino Acid Contents of Some Food Proteins from the Corresponding Amino Acid Contents of Whole Egg Proteins Taken as a Standard (15).

Amino Acid	Amino Acid Content of Whole Egg Proteins %	Percentage Deviation from Corresponding Values for Whole Egg Proteins											
		Cow's Milk	Casein	Lactal-bumin	Beef Muscle	Blood Serum Albumin	Blood Globulin	Blood Fibrin	Whole Wheat	White Flour	Whole Maize	Rolled Oats	Garden Peas
Arginine	6.4	—33	—34	—39	+20	—6	—25	+22	—34	—39	—25	—6	+39
Histidine	2.1	+24	+43	0	+38	+67	+19	+29	0	+5	—5	+5	—43
Lysine	7.2	+4	+10	+33	+12	+44	—7	+22	—63	—72	—72	—54	—31
Tyrosine	4.5	+18	+53	—2	—24	+18	+51	+29	—2	—16	+22	+2	—
Tryptophan	1.5	+7	—20	+67	—13	—80	+93	+127	—20	—33	—47	—20	—53
Phenylalanine	6.3	—10	—11	—14	—22	+22	—	—5	—10	—13	—21	+5	—24
Cystine	2.4	—58	—87	+71	—46	+171	+29	—21	—25	—21	—37	—25	—50
Methionine	4.1	—17	—15	—34	—20	—68	—73	—24	—39	—63	—24	—41	—76
Cystine + Methionine	6.5	—32	—42	+5	—29	+5	—35	—23	—34	—48	—29	—35	—66
Threonine	4.9	—8	—16	+10	—6	+4	—	+61	—33	—45	—24	—29	—20
Leucine	9.2	+23	+8	+13	—16	+29	—12	+52	—26	—18	+139	—10	—30
Isoleucine	8.0	+6	—19	—20	—21	—75	—59	—37	—55	—54	—50	—30	—49
Valine	7.3	+15	—8	—12	—21	—4	+38	—18	—38	—42	—32	—14	—45
Amino acid indicated as limiting	—	cystine + methionine	cystine + methionine	methionine	cystine + methionine	tryptophan	methionine	isoleucine	lysine	lysine	lysine	lysine	methionine

From this table it can be seen, for example, that the cereal and grain proteins are deficient in several amino acids, particularly lysine. On the other hand milk, although low in cystine and methionine, provides more than its share of many of the amino acids—especially lysine, tryptophan, isoleucine and valine. Thus, a combination of milk proteins and cereal proteins (as milk and cereal, or as bread enriched with milk solids) constitutes an excellent mutually supplementing combination providing a well-balanced protein mixture.

The supplementary value of whole milk, skim milk solids and cheese proteins to bread and potatoes has been effectively demonstrated by biological studies with experimental animals (16, 17). Similar supplementation benefits have been found for beef and cereal protein mixtures. Other studies with rats have shown marked benefits from the supplementation of white bread with skim milk solids, yeast proteins or, better still, with both as determined by food consumption levels, weight and food utilization (18). As a matter of fact, bread enriched with 6% dry non-fat milk solids was found by Mitchell and coworkers (18a) to be superior even to bread enriched with thiamine, riboflavin, nicotinic acid and iron. The use of dry milk solids improved the nutritive value not only of white bread but of whole wheat bread as well. These findings indicate that enrichment of bread with vitamins and iron is not the whole story; the added value of non-fat milk solids may well be attributed to its protein supplementing value and, possibly, to other unidentified nutrients present in milk. On the other hand adding whole wheat to white flour to improve the protein value would be quite ineffective because the limiting amino acid deficiency in each is lysine; no supplementation would occur.

In order to obtain the most information about any individual protein food, or mixture of foods, therefore, it is thus necessary to know the essential amino acid composition of the food or of the entire mixture. Deductions drawn from an analysis of these data should then be confirmed by biological animal tests. Promising as the chemical method is, it is, nevertheless, still necessary to rely on biological studies for confirmation. This is largely because of the fact that as a result of commercial food processing methods, heat-treatment, etc., certain essential amino acids may become metabolically non-utilizable even though they are still demonstrable by amino acid analysis.

The Time Factor in Supplementation:

The importance of protein supplementation as an effective means of providing nutritionally adequate protein mixtures in both normal and therapeutic

diets had scarcely been demonstrated before a revolutionary new concept made it imperative to revise methods for the practical application of this dietary principle. Up until fairly recently it was commonly believed that successful protein supplementation could be achieved by eating an incomplete protein or protein mixture at one meal and supplementing its deficiencies with a suitable protein at the next meal. Recent researches have shown, however, that this is by no means the case. It has been found that protein synthesis in the body and a retention of nitrogen for growth necessitate the *simultaneous* presence of *all* of the essential amino acids.

This concept was foreshadowed as early as 1929 in the work of Berg and Rose (19) who observed that when rats were fed a tryptophan-free ration, growth was improved when the tryptophan was administered at frequent intervals. A further clue was elicited some ten years later through the studies of Elman on protein hydrolysates.* He found (20) that when an acid hydrolyzed protein (in which tryptophan is destroyed) was given to dogs positive nitrogen balance occurred only if the missing amino acid was given together with the hydrolysate. If no tryptophan supplement was given, or if tryptophan was given separately several hours after the hydrolysate, a negative nitrogen balance resulted.

In 1946, this concept received new emphasis through the study of Melnick, Oser and Weiss (21) on the rate of protein digestion as a factor in nutrition. They suggested that for optimal utilization of food proteins all essential amino acids must not only be available for absorption but must also be liberated during digestion *in vivo* at rates permitting effective mutual supplementation. A year later, Geiger (24) showed that amino acid mixtures deficient in either tryptophan, methionine or lysine failed to promote growth. When the missing amino acid was added to the mixture growth ensued. When, however, the missing amino acid was fed *several hours* after the incomplete amino acid mixture, no supplemental effect could be demonstrated and growth failed to occur. As a result of these and other experiments (26) it was shown that amino acids are not stored in the body either as such or as peptide fractions, but are irreversibly further metabolized and are, therefore, not available for future protein synthesis. For protein synthesis, it was found, *all the essential* components have to be present simultaneously.

Just about this time, Cannon *et al* (25) reported that protein-depleted rats recovered weight steadily when fed an adequate diet in which the only

*cf. *Borden's Review of Nutrition Research,* Dec. 1945, "Protein Hydrolysates in Nutritional Emergencies I. Hypoproteinemia."—Jan. 1946, "Protein Hydrolysates in Nutritional Emergencies II. Clinical Studies."

source of protein was a mixture of the ten essential amino acids. If, however, the basal ration was divided into two portions, one containing five of the amino acids and the other containing the remaining five—*and the two portions fed alternately* — poor weight recovery or even continued weight loss was obtained. This occurred even when the two rations were offered at alternate hours over a 14-hour period followed by the non-protein basal ration for the rest of the 24-hour period. When, on the other hand, the two incomplete rations were combined and fed under similar alternating conditions, weight recovery was rapid. The authors concluded, like Geiger, that for effective tissue synthesis all of the essential amino acids must be available in the tissues practically simultaneously; otherwise the first group of amino acids absorbed is not stored long enough to enable them to combine with the amino acids of the second group for the synthesis of complete tissue proteins. Findings of a somewhat similar nature were reported soon after by still other investigators (27, 28, 29).

Time Studies with Proteins: Because of all the studies just described involved amino acids or mixtures of amino acids, the interpretation of results is somewhat restricted in terms of practical dietary conditions. The proteins actually present in foods differ from the amino acids in that they are slowly digested and absorbed. Accordingly, Geiger (30) attempted to determine whether the findings with amino acids could be extended to dietary proteins. Accordingly, he selected pairs of proteins which, fed separately, do not promote growth but which, when given together, supplement each other's amino acid deficiencies. These protein pairs were then fed alternately in a duplication of the earlier experiments on the delayed supplementation of amino acids. Thus, he showed that animal diets containing wheat gluten plus blood protein, or yeast plus blood protein, or yeast plus wheat gluten protein promote satisfactory growth. Growth did not result, however, when these same pairs of proteins were fed separately during alternating 10-hour periods with two-hour intervals between feedings. As a result of these experiments, he was able to demonstrate that delayed provision of the missing essential amino acids is ineffective, not only when fed as the amino acids themselves, but also when they are supplied in the form of proteins.

Similar findings were reported in animal studies by Henry and Kon (16). They noted that the supplementary relationship of milk and potato and of bread and cheese did not exist if these foods were fed separately on alternate days but could be demonstrated only if the two foods were fed together.

Human Studies:

In view of these findings with experimental animals, the possibility exists that the nitrogen metabolism of human subjects also may be influenced by the time of ingestion of proteins, especially with respect to the distribution of complete proteins among the usual daily meals. As Leverton and Gram (31) point out, if one meal such as breakfast is completely devoid of a high quality complete protein, there is the possibility that the nitrogen utilization of the individual will be impaired even though the total daily intake of protein is adequate. In a preliminary investigation of this problem they compared, under controlled conditions, (a) the daily nitrogen metabolism of fourteen college girls (ages 17 to 23) when the amount of protein in the diet was adequate, but no animal protein was included in the breakfast with (b) the nitrogen metabolism of the same subjects on the same protein intake when breakfast included some food which furnished a high quality protein.

The experimental period was divided into two parts of 18 days each, period A (no animal protein at breakfast) and period B (milk added to the breakfast). The difference between period A and B was solely in the *distribution* of the animal protein (milk)—the quality and total daily quantity remained the same. During period A, 8 oz. of milk were served at the noon meal, 16 oz. at the evening meal and none at breakfast. During period B, the 24 oz. of milk were distributed equally, i.e. 8 oz. at each of the three meals.

Striking differences in nitrogen metabolism during the two periods were noted. In every case, urinary excretion of nitrogen was less and percentage retention of nitrogen was greater in period B than during period A. Assuming these criteria to be indicative of better protein utilization, the authors conclude that there was an advantage in increasing the amount of protein at breakfast and providing animal protein at every meal.* They state, therefore, that in view of the nature and significance of these findings it is possible that the distribution of quantity and kind of protein in the three meals, as well as the total daily amount of protein, must be considered in determining protein requirements and utilization.

---

*The authors point out that there are many other factors not included in this preliminary study which must be considered. For example, they increased the quantity as well as quality of the breakfast protein (breakfast consisted of citrus fruit juice; bread made with water, not milk; butter, jam and coffee or Postum), although the total daily protein remained constant. The results might have been different if the protein content of the breakfast had remained constant, or if it had been increased with plant protein. With lunch at noon and dinner at 6 p.m., all the animal protein was eaten within 6-7 hours, leaving a period of 17-18 hours when no animal protein was taken into the body. The nitrogen metabolism might have been different if animal protein had been given for breakfast and dinner instead of for lunch and dinner. Because of the time factor, lack of animal protein in the breakfast may impair nitrogen utilization more than its lack in the mid-day meal. These questions are being investigated further.

Conclusion:

To the dietitian, public health worker and other food specialists engaged in translating nutrition research into everyday practical dietetics, the modern concept of protein supplementation has proved a most useful and resourceful tool.

The practical application of the supplementation phenomenon will, of course, necessitate some revision in methods of menu planning. *Each meal* will have to be planned to provide a total protein mixture containing adequate amounts of all the essential amino acids. Protein foods can no longer be evaluated singly in terms of nutritive value, but must be viewed in the light of their possible supplemental value to the other proteins of the diet —in other words, as one fragment of a composite total. In addition, new problems will be posed in the estimation of dietary intake via the nutrition survey method. In order to establish the protein adequacy of a diet from survey data and diet histories, it will be necessary to know not only the specific proteins eaten, but the combinations of these which were used, their distribution throughout the daily meals and the time interrelationships which existed with respect to their intake.

Through the use of this new nutrition tool it will become possible to plan diets around cheap and abundant foodstuffs. Such foods although frequently of inferior protein quality, can be made the nutritional equivalent of expensive or scarce top quality protein foods by combining them with suitable amounts of supplementary proteins to compensate for their amino acid deficits. The adaptation of these principles to large-scale world food shortage and feeding problems constitutes one of the major contributions of modern nutrition to human welfare.

REFERENCES CITED

1. Rose, W. C. The nutritive significance of the amino acids. *Physiol. Rev. 18,* 109 (1938).

2. Rose, W. C. Human amino acid requirements. *Fed. Proc. 8,* 546 (1949).

3. Work, Elizabeth. A new naturally occurring amino acid. *Nature 165,* 74 (1950).

4. Osborne, T. B., L. B. Mendel and E. L. Ferry. Method of expressing numerically the growth promoting value of protein. *J. Biol. Chem. 37,* 223 (1919).

5. Barnes, R. M., J. E. Maack, M. J. Knights and G. O. Burr. Measurement of growth promoting quality of dietary protein. *Cereal Chem. 22,* 273 (1945).

6. Bosshardt, D. K., L. C. Ydse, M. M. Ayres and R. H. Barnes. The use of mice for measurement of the growth promoting quality of proteins. *J. Nutr. 31,* 23 (1946).

7. Mitchell, H. H. Method of determining biological value of proteins. *J. Biol. Chem. 58,* 873 (1924).

8. Mitchell, H. H. and G. G. Carman. Biological value of nitrogen of mixtures of patent white flour and animal foods. *J. Biol. Chem. 68,* 183 (1926).

9. MITCHELL, H. H., T. S. Hamilton, J. R. Beadles and F. Simpson. The importance of commercial processing for the protein value of food products. *J. Nutr. 29,* 13 (1945).

10. MITCHELL, H. H., W. Burroughs and J. R. Beadles. The significance and accuracy of biological values of proteins computed from nitrogen metabolism data. *J. Nutr. 11,* 257 (1936).

11. MITCHELL, H. H. Biological methods of measuring protein values of feeds. *J. Animal Sci. 2,* 263 (1942).

12. MITCHELL, H. H. Determination of the nutritive value of the proteins of food products. *Ind. Eng. Chem. Anal. Ed. 16,* 696 (1944).

13. MITCHELL, H. H. and R. J. Block. Some relationships between the amino acid contents of proteins and their nutritive values for the rat. *J. Biol. Chem. 163,* 599 (1946).

14. SUMNER, E. E. The biological value of milk and egg protein in young and mature rats. *J. Nutr. 16,* 129 (1938).

15. BLOCK, R. J. and H. H. Mitchell. The correlation of the amino acid composition of proteins with their nutritive value. *Nutr. Abstr. and Rev. 16,* 249 (1946).

15a. OSER, B. 112th Meeting, American Chemical Society, April (1950).

16. HENRY, KATHLEEN M. and S. K. Kon. The supplementary relationships between the proteins of dairy products and those of bread and potato as affected by the method of feeding. *J. Dairy Res. 14,* 330 (1946).

17. HENRY, KATHLEEN M., S. K. Kon and S. J. Rowland. Studies of the biological value of the proteins (nitrogen) of dried skim milk: Value as an addition to bread; etc. *J. Dairy Res. 14,* 403 (1946).

18. SEELEY, R. D., H. F. Ziegler, Jr. and R. J. Sumner. The nutritional value of white bread supplemented with non-viable dried yeast. Address before the Amer. Assoc. of Cereal Chemists, N. Y. C., May (1949).

18a. MITCHELL, H. H., T. S. Hamilton and J. B. Shields. The contribution of non-fat milk solids to the nutritive value of wheat breads. *J. Nutr. 25,* 585 (1943).

19. BERG, C. P. and W. C. Rose. Tryptophan and growth: growth upon a tryptophan-deficient basal diet supplemented at varying intervals by the separate feeding of tryptophan. *J. Biol. Chem. 82,* 479 (1925).

20. ELMAN, R. Time factor in retention of nitrogen after intravenous injection of mixture of amino acids. *Proc. Soc. Exp. Biol. Med. 40,* 484 (1939).

21. MELNICK, D., B. L. Oser and S. Weiss. Rate of enzymic digestion of protein as a factor in nutrition. *Sci. 103,* 326 (1946).

22. PADER, M., D. Melnick and B. L. Oser. Factors effecting the availability of lysine in heat-processed casein. *J. Biol. Chem. 172,* 763 (1948).

23. MELNICK, D. and B. L. Oser. The influence of heat processing on the functional and nutritive properties of proteins. *Food Tech. 3,* 57 (1949).

24. GEIGER, E. Experiments with delayed supplementation of incomplete amino acid mixtures. *J. Nutr. 34,* 97 (1947).

25. CANNON, P. R., C. H. Steffee, L. J. Frazier, D. A. Rowley and R. C. Stepto. The influence of time of ingestion of essential amino acids upon utilization in tissue synthesis. *Fed. Proc. 6,* 390 (1947).

26. GEIGER, E. *Physiology of the Utilization of Dietary Amino Acid Mixtures.* Dissertation. Univ. So. Calif., Los Angeles (1947).

27. HARTE, R. A., J. J. Travers and P. Sarich. The effect on rat growth of alternated protein intakes. *J. Nutr. 35,* 287 (1948).

28. PEARCE, E. I., H. G. Sauberlich and C. A. Baumann. Amino acids excreted by mice fed incomplete proteins. *J. Biol. Chem. 168,* 271 (1947).

29. YESHODA, K. M. and M. Damodaran. Amino acids and proteins in hemoglobin formation. *Biochem. J. 41,* 382 (1947).

30. GEIGER, E. The role of the time factor in feeding supplementary proteins. *J. Nutr. 36,* 813 (1948).

31. LEVERTON, R. M. and M. R. Gram. Nitrogen excretion of women related to the distribution of animal protein in daily meals. *J. Nutr. 39,* 57 (1949).